

# **CONDITIONED MEDIUM FROM CELLS CULTURED UNDER HYPOXIC CONDITIONS AND USES THEREOF**

## **FIELD**

**[0001]** The present invention relates to the production and use of extracellular matrix or conditioned medium compositions and more specifically to compositions obtained by culturing cells under hypoxic conditions on a surface in a suitable growth medium. Use of the composition is also contemplated.

## **BACKGROUND OF THE INVENTION**

**[0002]** Conditioned medium from in vitro studies and their use in a variety of therapeutic and medical applications have been described in the art. One therapeutic application of extracellular matrix (ECM) compositions includes the treatment and repair of soft tissue and skin defects such as wrinkles and scars.

**[0003]** The conditioned medium compositions may include amino acids, salts, vitamins, minerals sugars, lipids, nucleosides and proteins. The conditioned medium may contain many of the original components of the cell culture medium as well as a variety of cellular metabolites and secreted proteins including, for example, biologically active growth factors, inflammatory mediators and other extracellular proteins.

**[0004]** The repair or augmentation of soft tissue defects caused by acne, surgical scarring or aging has proven to be very difficult. A number of materials have been used to correct soft tissue defects with varying degrees of success, however, no material has been completely safe and effective. For example, silicon causes a variety of physiological and clinical problems including long term side effects such as nodules, recurring cellulitis and skin ulcers.

**[0005]** Accordingly, new materials are needed for soft tissue repair and treatment of soft tissue that may overcome the deficiencies of prior materials.

**[0006]** In vitro cultured medium can also be used to treat damaged soft tissue such as dermal tissue, for example.

**[0007]** In vitro cultured medium can additionally be used to repair and/or regenerate damaged cells or tissue such as dermal tissue, for example. The compositions of the present invention may be useful for treatment of trauma to the skin such as burns, laser treatment, and the effects of aging. Effects of aging may include skin discoloration, uneven skin texture, roughness and uneven skin tone, for example.

**[0008]** Fueled in part by the stem cell revolution, tissue engineering technology offers the promise of tissue regeneration and replacement following trauma or treatment of degenerative diseases. It can also be used in the context of cosmetic procedures.

**[0009]** Tissue engineering techniques can be used to generate both autologous and heterologous tissue or cells using a variety of cell types and culture techniques. In creating an autologous implant, donor tissue may be harvested and dissociated into individual cells, and subsequently attached and cultured on a substrate to be implanted at the desired site of the functioning tissue. Many isolated cell types can be expanded in vitro using cell culture techniques, however, anchorage dependent cells require specific environments, often including the presence of a three-dimensional scaffold, to act as a template for growth.

**[0010]** Thus, the development of natural materials that are suitable for topical application is greatly needed.

## **SUMMARY**

**[0011]** The embodiments herein encompass methods and compositions comprising human fibroblast-derived cell culture medium that is cultured under hypoxic conditions.

**[0012]** In a first aspect, a method of producing a composition for improvement of tissue in a subject is provided, the method comprising: culturing human fibroblast cells under hypoxic conditions on microcarrier beads or a three dimensional surface in a suitable cell culture medium under 1-5% oxygen, thereby simulating the early embryonic environment, and generating a composition with embryonic-like proteins that promotes the repair and regeneration of damaged tissue when administered to the region of tissue in need of repair. In some embodiments, the method further comprises adding at least one botanical to the composition. In some embodiments, the method further comprises adding at least one extract to the composition. In some embodiments, the method further comprises adding at least one peptide to the composition. In some embodiments, the culturing is performed on microcarrier beads or a three-dimensional surface in a suitable cell culture medium. In some embodiments, the culturing is performed for at least two weeks. In some embodiments, the conditioned medium is collected after two weeks. In some embodiments, the method further comprises adding a seed extract to the composition. In some embodiments, the method further comprises adding a marine extract to the composition. In some embodiments, the method further comprises adding a bacterial ferment to the composition. In some embodiments, the conditioned medium has embryonic-like proteins. In some embodiments, the conditioned medium further comprises cytokines. In some embodiments, the conditioned medium further comprises at least one matrix protein. In some embodiments, the at least one peptide comprises dimer tripeptide 43 and/or trifluoroacetyl tripeptide-2. In some embodiments, the subject is in need of tissue repair. In some embodiments, the subject has fine and or deep wrinkles. In some embodiments, the subject exhibits tactile and/or skin roughness of the tissue. In some embodiments, the subject has hyperpigmentation. In some embodiments, the subject has photodamage. In some embodiments, the subject lacks evenness in pigmentation and/or skin tone. In some embodiments, the subject has a skin coloring on the Fitzpatrick scale of 1, 2, 3, 4 or 5.

**[0013]** In a second aspect, a composition made by the method of any one of the embodiments described herein is provided for use in treating a subject.

**[0014]** In a third aspect, a composition is prepared from a conditioned medium that is prepared from a cell culture medium cultured with embryonic-like proteins under hypoxic conditions, at least one botanical, at least one extract and at least one peptide. In some embodiments, the composition is odorless. In some embodiments, the composition is clear.

**[0015]** In a fourth aspect, a method of improving the appearance of an individual is provided, the method comprising: topically applying a composition of any one of the embodiments described herein onto the surface of a subject's skin to thereby improve the aesthetic quality of the skin. In some embodiments, the subject has fine or deep wrinkles. In some embodiments, the subject has tactile